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STUDIES IN SULFOFICATION

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Recent experiments [1] have shown that sulfofication or sulfur-oxidation is an important process occurring in field soils. Plants have been found to require considerable amounts of sulfates for their best growth and inasmuch as sulfur is not present in soils in that form, but as unassimilable organic and inorganic compounds, the sulfur-feeding of crops will depend very largely upon the ability of a soil on which they grow to produce sulfates from these unavailable substances. In other words, the total sulfur content of a soil, alone, will not show the sulfur available for plant growth. The sulfofying power of the soil must also be ascertained.

The investigations mentioned, besides demonstrating the fact that all soils possess a definite sulfofying power which is determinable in the laboratory, threw considerable light on the conditions governing the process. Thus it was found that additions of green manure and barnyard manure increased the sulfofying power of the soil; also in general, that the treatment which the soil had undergone influenced considerably its ability to produce sulfates. Furthermore, the optimum moisture content of the soil for the process was found to be fifty per cent of the amount necessary for complete saturation, and the oxidation of sulfur was found to occur to the greatest extent in a mixture of fifty per cent soil and fifty per cent sand, showing the importance of aeration. Again the addition of carbohydrates to the soil was shown to depress sulfofication, the greater the amount added, the greater the depression. The depression also varied in the inverse ratio to the solubility of the carbohydrates.

A definite laboratory method was devised for determining the sulfofying power of soils. This consisted in the addition of 0.1 gm. of Na_2S or free sulfur, preferably the latter, to 100 gm. quantities of fresh soil, adjustment of the moisture content to the optimum for the soil, and incubation for 5-10 days. The sulfates were then determined by shaking the soil with water for seven hours in the shaking machine, filtering, precipitating the sulfates with barium chloride, and estimating in the sulfur photometer.

Studies for the sulfur content of Iowa soils confirmed the observations of Hart and Peterson [2] in Wisconsin and Shedd [3] in

[1] Brown & Kellogg. Rsch. Bull. Iowa Agr. Expt. Sta. 19, 1914.
[2] Rsch. Bull. Wis. Agr. Expt. Sta. 14.
[3] Bull. Kentucky Agr. Expt. Sta. 174.

Kentucky, that much less sulfur on the average than phosphorus was present in the various large soil areas. Some of the sulfur removed from the soil by crops may, of course, be returned by the use of manure, but the manure produced on a livestock farm is generally insufficient to keep up the sulfur content of the soil, and unless manure is purchased or large amounts of commercial feeds are used, commercial sulfur-containing fertilizers must be applied to maintain the soils permanently fertile.

This does not mean that applications of sulfur fertilizers would prove profitable on Iowa soils at the *present time*, but it does mean that unless different methods of soil treatment are employed than those at present in use, sulfur will be lacking at *some future time*. For *permanent soil fertility*, the sulfur supply for crops must be considered.

The amount of phosphorus in Iowa soils is low and in many cases this element may be the limiting factor of growth. Acid phosphate and rock phosphate are the two materials which are available commercially for supplying phosphorus. The former supplies sulfur as well, and the question arises whether it has any superior value for that reason. The relative merits of the two phosphorus compounds are not yet definitely known and it is possible that the sulfur content and also the effect on sulfofication should be considered in selecting the material for remedying deficiencies of phosphorus in soils.

The relative effects of the materials mentioned on sulfofication, on ammonification, and on crop yields should also be ascertained. If sulfofication and ammonification run parallel, it will be evident that methods of treatment which stimulate nitrate production will also lead to greater sulfate production. If crop yields and sulfofication are similarly affected, it may be that the effects of the materials are largely due to the sulfur factor. The following experiment was planned therefore to throw some light on the problem of the relative effects of gypsum, acid phosphate, rock phosphate alone and with gypsum, and mono-calcium phosphate on sulfofication, on ammonification and on the yields of oats in pots in the greenhouse.

THE PLAN OF THE EXPERIMENT

The soil used in this work was a black loam, high in organic matter and having a basic reaction. When analyzed, it was found to contain 911 lbs. of phosphorus and 2,487 lbs. of sulfur per acre of 2,000,000 lbs. of surface soil. This sulfur content was very high, much higher than that of any of the samples of Iowa soils whose analyses were given in the bulletin already referred to.

The results secured were undoubtedly modified considerably because of the presence of so much sulfur in the soil used. The use of gypsum, for instance, could hardly be expected to show any effect and all of the applications would probably exert much more influence on sulfofication in a soil lower in sulfur.

This soil was evidently somewhat abnormal for Iowa conditions, and hence the results should not be interpreted as of more than technical interest.

Twenty-four four-gallon pots were filled with the soil described which was air-dried, 27.6 lbs. being placed in each pot.

The special treatments were as follows:

Pots	*Treatment*
1–2	Check.
3–4	24.7 lbs. calcium sulfate per acre.
5–6	70.5 lbs. mono-calcium phosphate per acre.
7–8	300 lbs. acid phosphate per acre.
9–10	1000 lbs. rock phosphate per acre.
11–12	1000 lbs. rock phosphate+24.7 lbs. $CaSO_4$ per acre.
13–14	Check.
15–16	24.7 lbs. $CaSO_4$ per acre.
17–18	70.5 lbs. mono-calcium phosphate per acre.
19–20	300 lbs. acid phosphate per acre.
21–22	1000 lbs. rock phosphate per acre.
23–24	1000 lbs. rock phosphate+24.7 lbs. $CaSO_4$ per acre.

Pots 13 to 24 were seeded to oats and the remainder were kept bare for bacteriological tests.

The applications were based on actual field conditions, the 300 lbs. of acid phosphate and 1,000 lbs. of rock phosphate forming the basis of the additions. The acid phosphate was analyzed for phosphorus and sulfur and showed a content of 5.2 per cent of phosphorus and 1.994 per cent of sulfur. The applications of calcium sulfate and mono-calcium phosphate made to the soil were calculated so that the same amounts of sulfur and phosphorus should be added, respectively, as were applied in the acid phosphate.

The rock phosphate contained 5.85 per cent of phosphorus and hence considerably more phosphorus was applied than in the case of the acid phosphate, but the amounts of both materials used were those commonly employed on the farm and a fair comparison was provided. The variation in available phosphorus, of course, accounts for the difference in the amounts applied. The optimum moisture content of the soil was determined and after the pots were filled, distilled water was applied to bring all the soils up to that content. The pots were then weighed, and during the continuance of the experiment the moisture content was maintained by additions of distilled water to weight.

The oats were harvested just prior to maturity, the green and dry weights secured and the nitrogen content determined. Samples were drawn for bacteriological tests every two weeks, the sulfates present were determined, the moisture content ascertained and tests for sulfofying power by the free-sulfur-fresh-soil method previously described and for ammonification by the casein-fresh-soil method and the dried blood-fresh-soil method were carried out. The usual precautions were observed in sampling to secure uncontaminated samples. The sulfate determinations were made by shaking the soil with water for seven hours in the shaking machines as usual. The ammonia determinations were made by the magnesium-oxide method.

The experiment was begun on January 11, and the samples were drawn on January 26, February 9, February 23, March 9 and March 23.

THE SULFATES PRESENT AT SAMPLING

The sulfates present in the soils at the various samplings are given in table I and the average content under the different treatments are calculated.

There was little variation in sulfate content at the different samplings. The amounts added were very small and evidently the method used in the subsequent determinations was not sufficiently accurate to show them.

There are some indications at all the samplings, except the last, of a depression in sulfate content in the treated soils, but the differences were too small to be distinctive. The later sulfo-

TABLE I. SULFUR AS SULFATES PRESENT AT TIME OF SAMPLING

Pot No.	Lab. No.	1st Samples		2nd Samples		3rd Samples		4th Samples		5th Samples	
		Mgs. S. as SO_4	Av. for Treat-ment	Mgs. S. as SO_4	Av. for Treat-ment	Mgs. S. as SO_4	Av. for Treat-ment	Mgs. S. as SO_4	Av. for Treat-ment	Mgs. S. as SO_4	Av. for Treat-ment
1	1	4.98		6.22		5.95		5.20		5.36	
	2	5.07		7.27		5.81		5.71		4.48	
2	3	4.88		7.27		6.87		6.53		6.68	
	4	5.17	5.02	7.27	7.00	7.28	6.50	6.29	5.93	6.53	5.75
3	5	3.91		5.81		6.36		5.88		5.84	
	6	4.75		5.61		6.36		6.12		6.92	
4	7	3.87		5.40		5.61		5.88		5.36	
	8	5.17	4.43	5.71	5.63	5.71	6.01	5.61	5.87	5.52	5.91
5	9	4.83		6.36		7.21		6.12		6.84	
	10	4.83		7.82		7.14		6.46		6.56	
6	11	4.54		5.61		5.11		4.93		5.68	
	12	5.17	4.84	5.34	6.28	5.71	6.44	4.59	5.52	5.68	6.19
7	13	4.70		5.27		6.87		5.24		6.28	
	14	5.37		5.44		6.02		5.78		6.60	
8	15	4.83		4.56		5.34		4.83		6.12	
	16	5.17	5.02	4.83	5.02	5.27	5.89	5.24	5.27	6.33	6.33
9	17	3.64		4.25		4.56		4.69		5.52	
	18	4.39		4.18		4.49		4.69		5.12	
10	19	3.51		4.25		4.39		4.96		5.36	
	20	4.70	4.05	4.35	4.26	4.56	4.49	4.76	4.77	5.52	5.38
11	21	4.99		5.27		6.46		7.07		6.84	
	22	5.17		5.44		6.36		6.60		6.84	
12	23	3.79		5.40		6.53		5.20		5.76	
	24	4.88	4.71	5.40	5.37	6.38	6.43	5.44	6.07	6.08	6.38

fying tests showed increases in sulfofying power, due to treatment, and hence it would hardly be reasonable to assume any depression in sulfate content in this case. The variations in results should, therefore, probably be regarded as due to the method of determination and as indicating the absence of any effect of the materials added, rather than as distinctive differences.

The variations in sulfate content from one sampling to the next were very slight, much smaller than is usually the case with nitrates. There are such variations, however, that the conclusion seems justified that sulfate production and assimilation vary in much the same way that nitrate production and assimilation vary. That is, there may be an accumulation up to a certain point which is followed by increased assimilation and hence a decrease in the amount present. In the field, of course, there are losses of sulfates by leaching and assimilation by plants, just as in the case of nitrates, but in these experiments there was no leaching and no plants grown and hence the differences were due to variations in production and assimilation by bacteria. There are evidently certain sulfate-assimilating bacteria which may become very active in the presence of abundance of sulfates and whose activity declines as the sulfates are used up.

It will be left for future experiments to learn more of these sulfate-assimilating bacteria. Their activities may be a source of removal of sulfates from the use of crops, but it is more probable that they should be regarded as a means of preserving sulfates in the soil and preventing the loss by leaching. Sulfates which are used by the assimilating bacteria would later become available again for plant growth and hence at times of too large sulfate production for the needs of crops these bacteria would prove of much value in preventing losses by leaching.

It is evident also from these results that sulfates do not accumulate in soils any more than nitrates do. They seem to be subject to much the same influences as nitrates and this fact suggests that sulfate production and also sulfate assimilation are very closely related, respectively, to nitrate production and nitrate assimilation and that the influence of certain known factors on the nitrogen processes may be the same on the sulfur processes.

THE SULFOFICATION TESTS

The samples drawn on the dates given previously, were tested for their sulfofying power according to the method described — the free-sulfur-fresh-soil method.

The results secured at the various samplings are given in tables II, III, IV, V, and VI and the average results for the different treatments appear in table VII.

Examining the results given in the complete tables it is found that the duplicate determinations agreed very closely, which shows that the method employed in the estimation of the sulfates was quite satisfactory.

TABLE II. PERCENT OF ADDED SULFUR, SULFOFIED, 1ST SAMPLES

Pot No.	Lab. No.	Mgs. S. as SO_4	Av. Mgs. S. as SO_4	Mgs. S. as SO_4 in soil after incubation	Av. Mgs. as SO_4	Mgs. S. as SO_4 from S. added	Percent S. Sulfofied for each treatment
1	1	lost		7.75			
	2	lost		7.36	7.55		
2	3	38.76		8.46			
	4	36.72	36.74	8.23	8.33	28.40	28.4
3	5	43.69		7.47			
	6	42.84	43.26	7.47	7.47	35.79	
4	7	43.69		7.34			
	8	45.90	44.78	7.34	7.34	37.44	36.6
5	9	40.46		7.92			
	10	42.50	41.48	8.09	8.00	33.48	
6	11	48.62		7.27			
	12	41.48	45.05	7.41	7.34	37.71	35.6
7	13	42.50		7.75			
	14	41.99	42.24	7.75	7.75	34.49	
8	15	38.76		5.88			
	16	36.38	37.57	5.57	5.72	31.85	33.2
9	17	43.69		5.71			
	18	41.16	42.41	5.71	5.71	36.70	
10	19	32.64		5.27			
	20	32.64	32.64	5.44	5.35	29.29	33.0
11	21	27.06		7.27			
	22	36.38	36.72	7.58	7.42	29.30	
12	23	36.04		6.22			
	24	34.68	35.36	6.35	6.28	29.08	29.2

TABLE III. PERCENT OF ADDED SULFUR, SUFOFIED, 2ND SAMPLES

Pot No.	Lab. No.	Mgs. S. as SO_4	Av. Mgs. S. as SO_4	Mgs. S. as SO_4 in soil after incubation	Av. Mgs. as SO_4	Mgs. S. as SO_4 from S. added	Percent S. Sulfofied for each treatment
1	1	32.30		7.99			
	2	31.11	31.70	8.19	8.09	23.16	
2	3	28.07		7.99			
	4	29.75	28.91	8.74	8.36	20.25	21.70
3	5	31.11		7.28			
	6	31.11	31.11	7.21	7.24	23.87	
4	7	35.19		6.97			
	8	36.89	36.04	6.87	6.92	29.12	26.54
5	9	35.19		8.02			
	10	35.53	35.36	8.40	8.21	27.15	
6	11	35.19		5.75			
	12	36.38	35.78	5.75	5.75	30.03	28.6
7	13	28.07		7.28			
	14	30.60	29.33	7.07	7.17	22.16	
8	15	28.90		6.29			
	16	26.69	27.79	5.88	6.08	21.71	21.9
9	17	30.09		5.24			
	18	31.11	30.60	5.20	5.22	25.38	
10	19	31.79		5.34			
	20	29.24	30.01	5.05	5.19	24.82	25.1
11	21	27.20		6.46			
	22	34.85	31.02	6.53	6.49	24.52	
12	23	27.71		6.73			
	24	31.79	29.75	6.29	6.50	23.25	23.9

TABLE IV. PERCENT OF ADDED SULFUR, SULFOFIED, 3RD SAMPLES

Pot No.	Lab. No.	Mgs. S. as SO_4	Av. Mgs. S. as SO_4	Mgs. S. as SO_4 in soil after incubation	Av. Mgs. as SO_4	Mgs. S. as SO_4 from S. added	Percent S. Sulfofied for each treatment
1	1	33.66		6.02			
	2	33.32	33.49	5.88	5.95	27.54	
2	3	34.85		6.60			
	4	34.85	34.85	6.60	6.60	28.25	27.9
3	5	39.10		7.28			
	6	39.61	39.35	6.97	7.12	32.23	
4	7	43.69		5.71			
	8	35.70	39.65	5.88	5.79	33.86	33.0
5	9	34.34		7.44			
	10	35.36	34.85	7.28	7.36	27.49	
6	11	31.79		5.17			
	12	33.66	32.72	5.34	5.25	27.47	27.5
7	13	32.30		5.20			
	14	31.96	32.13	4.42	4.81	27.32	
8	15	34.34		4.69			
	16	33.66	34.00	5.13	4.91	29.09	28.2
9	17	37.23		4.79			
	18	35.36	36.29	4.69	4.74	31.55	
10	19	41.99		5.00			
	20	38.76	40.37	4.83	4.91	35.46	33.5
11	21	39.95		5.85			
	22	41.99	40.92	6.19	6.02	34.90	
12	23	32.30		5.95			
	24	34.00	30.15	5.95	5.95	27.20	31.0

TABLE V. PERCENT OF ADDED SULFUR SUFOFIED, 4TH SAMPLES

Pot No.	Lab. No.	Mgs. S. as SO_4	Av. Mgs. S. as SO_4	Mgs. S. as SO_4 in soil after incubation	Av. Mgs. as SO_4	Mgs. S. as SO_4 from S. added	Percent S. Sulfofied for each treatment
1	1	34.6		6.92			
	2	34.2	34.4	6.92	6.92	27.5	
2	3	33.6		5.48			
	4	34.4	34.0	5.76	5.62	28.4	27.9
3	5	31.6		6.20			
	6	32.0	31.8	6.40	6.30	25.5	
4	7	41.6		6.32			
	8	45.6	43.6	6.04	6.18	37.4	31.4
5	9	52.2		5.12			
	10	50.0	51.1	5.76	5.44	45.6	
6	11	43.2		5.08			
	12	44.2	43.7	4.96	5.02	38.7	42.1
7	13	48.8		6.88			
	14	46.6	47.7	6.84	6.86	40.8	
8	15	47.0		6.72			
	16	46.0	46.5	6.32	6.52	40.0	40.4
9	17	50.0		5.36			
	18	51.4	50.7	5.16	5.26	45.5	
10	19	47.0		5.40			
	20	43.8	45.4	5.40	5.40	40.0	42.7
11	21	35.0		7.32			
	22	42.0	39.5	6.92	7.12	32.4	
12	23	40.4		6.40			
	24	39.2	39.8	5.40	5.90	33.9	33.1

TABLE VI. PERCENT OF ADDED SULFUR, SULFOFIED, 5TH SAMPLES

Pot No.	Lab. No.	Mgs. S. as SO_4	Av. Mgs. S. as SO_4	Mgs. S. as SO_4 after incubation	Av. Mgs. as SO_4	Mgs. S. as SO_4 from S. added	Percent S. Sulfofied for each treatment
1	1	35.4		5.12			
	2	36.6	35.9	5.08	5.10	30.8	
2	3	45.6		5.72			
	4	44.6	45.1	5.72	5.72	29.4	30.1
3	5	36.6		5.88			
	6	47.6	48.1	6.16	6.02	41.1	
4	7	45.6		5.40			
	8	44.6	45.1	5.40	5.40	39.7	40.4
5	9	45.6		5.88			
	10	43.2	44.4	4.88	5.88	38.6	
6	11	50.0		5.00			
	12	47.6	48.8	4.96	4.98	43.8	41.1
7	13	40.4		6.24			
	14	40.4	40.4	6.04	6.14	34.3	
8	15	48.8		5.68			
	16	45.6	47.2	5.56	5.62	41.6	37.9
9	17	47.0		5.52			
	18	46.6	46.8	5.16	5.34	41.5	
10	19	45.6		5.16			
	20	43.8	44.7	5.04	5.10	39.6	40.5
11	21	45.6		7.68			
	22	47.6	46.6	6.44	7.06	39.5	
12	23	46.0		6.04			
	24	49.4	47.7	6.04	6.04	41.7	40.6

The results from the duplicate pots were not always in perfect agreement, but that is ever the case in greenhouse experiments. Differences in the location of duplicate pots, with reference to the glass seem to exert an important influence on the bacteriological results as well as on the crop yields secured in the greenhouse. Of course, the danger of accidental contamination in soils under such abnormal conditions as pertain in the greenhouse, the growth of algae which is frequently observed, and the occurrence of molds and possibly also of protozoans are to be considered. But in general the differences in the heat and light relations may account for many of the variations which are encountered.

The results secured in this work from the duplicate pots were as uniform as is usually the case and whatever the causes of the variations may be, it was impossible to ascertain them, and hence the average results must be considered as fairly accurate.

Each table gives the amounts of sulfates present in the soils after incubation and upon examining these results and comparing them with the amounts of sulfates which the soils contained at sampling, given in table I, it appears that the incubation of the soils for ten days brought about only very slight changes in the sulfate content of the soils. It is evident, therefore, that the amount of sulfates present in soils does not change to any great extent in short periods of time. In other words, in the absence of leaching and of assimilation by crops, somewhat of an equilibrium seems to be established between sulfate-production and sulfate-assimilation. At any rate, the sulfate content of soils under these conditions changes so slowly that tests made within short intervals of time do not seem to show any large differences.

Under field conditions it is quite probable that the differences would be much greater and would appear more quickly. In fact it seems extremely doubtful if an equilibrium, such as was found here, would be established under field conditions in the presence of the disturbing factors of leaching and assimilation by crops. Unless special treatments were followed it would be reasonable to expect that the sulfate content of soils would gradually decline and such is actually the case in the field. As the total sulfur content becomes less, the production of sulfates becomes slower, as has been shown in the sulfofication studies already referred to. Hence, under field conditions, instead of an equilibrium in sulfates, a gradual decline is found unless special treatments are followed.

Upon subtracting the sulfate content of the soils, after incubation, from the total amount of sulfates found in the tests, the remainder is calculated as percent sulfur sulfofied and these are the figures which show the sulfofying power of the soils.

Turning to table VII, which gives the average percentages of sulfur sulfofied, some interesting facts become evident.

In the first place it is found that the calcium sulfate, even in the small applications made, increased to a marked degree the sulfofying power of the soil. This increase occurred at every date of sampling and bears out the results secured in the earlier experiments already referred to, according to which calcium sulfate increased the sulfofying power of the soil to a large extent, in direct proportion to the size of the application. Of course, if the amount of sulfate applied were increased beyond a certain point it is probable that no further increase in sulfofication would occur and an actual depression might take place.

TABLE VII. PERCENT OF ADDED SULFUR, SULFOFIED FOR EACH TREATMENT AT EACH SAMPLING

Treatment	Samples				
	1st	2nd	3rd	4th	5th
1. Checks	28.4	21.7	27.9	27.9	30.1
2. 24.7 lbs. $CaSO_4$	36.6	26.5	33.0	31.4	40.4
3. 70.5 lbs. $CaH_4(PO_4)_2$	35.6	28.6	27.5	42.1	41.1
4. 300 lbs. Acid Phosphate	33.2	21.9	28.2	40.4	37.9
5. 1000 lbs. Raw Rock Phosphate	33.0	25.1	33.5	42.7	40.5
6. 1000 lbs. Raw Rock Phosphate plus 24.7 lbs. $CaSO_4$	29.2	23.9	31.0	33.1	40.6

The interesting feature of the present results is that very small amounts of gypsum, such as may be added to soils in another fertilizing material (acid phosphate), may exert a pronounced influence on the soil's ability to produce sulfates. Thus the effects of gypsum may be partly due to a stimulative action, as has been supposed, as well as to the addition of a plant food constituent. The stimulative action may be of considerable importance on soils which contain sufficient amounts of total sulfur but do not have a rapid enough sulfofying action. In other words, if soils are found which contain fairly large amounts of sulfur, but on which crops are not supplied with sufficient sulfates for their best growth, applications of small amounts of gypsum might be sufficient to stimulate sulfofication to such an extent that the sulfur already present in the soil would be sulfofied rapidly enough to keep plants supplied with that element.

The mono-calcium phosphate gave considerable increases in sulfofication, and these were especially pronounced at the last two samplings. The increases were very similar to those brought about by the gypsum, varying somewhat from those results as might be expected. It is apparent that this material exerted some stimulative action on sulfofication, and if it has any effect on crop growth, that effect might be considered to be due in part to an increased production of sulfates and not entirely to the phosphorus supplied. The action of this material may be somewhat indicative of the effect of acid phosphate, assuming that the phosphorus in this latter material is in the mono-calcic form, which it is, in part at least.

The applications of acid phosphate increased the sulfofying power of the soil, but to a smaller extent in practically all cases than either the gypsum or the mono-calcium phosphate alone. It appears, therefore, that on this soil the combination of the two substances was not as beneficial for sulfofication as either of them alone. Just why this should be the case is difficult to determine. It is probably, however, the result of more complicated bacterial changes brought about by the combined substances, although the other calcium phosphates present in the acid phosphate, such as the dicalcic and tricalcic phosphates, may explain the different effects.

An interesting practical point is brought out here, however, and that is that acid phosphate when applied to this soil had a stimulative action on sulfofication, and hence its influence on crop yields, if it exerts any whatever, may not be due entirely to the phosphorus which it supplies to the crops in available form or to the sulfate which is supplied, but in part to the increase in sulfate production from the soil. Previous suggestions regarding the value of acid phosphate because of effects on the sulfur feeding of plants are thus confirmed and it seems reasonable to conclude that on soils deficient both in phosphorus and sulfur acid phosphate would be a good material to use to supply both deficiencies, increasing the sulfates available for plants both by actual additions and by increased production in the soil.

Raw rock phosphate, applied at the rate of 1,000 lbs. per acre, a normal farm application, increased the sulfofying power of the soil to a greater extent than did the acid phosphate, also applied in the customary field amount. The increase was about the same as that exerted by the mono-calcium phosphate. Only one reason suggests itself in explanation of the greater influence of the rock phosphate over the acid phosphate and that is the greater amount of phosphorus used. Perhaps the sulfofying bacteria use phosphorus in their growth and the stimulative effect of phosphorus fertilizers on sulfofication is really due to a feeding of the sulfofiers. In such a case the question arises as to the form in which the phosphorus is required by the bacteria. Probably it must be soluble, when it would be expected that acid phosphate would give greater increases than rock phosphate. However, it may be that the rock phosphate was rendered available much more rapidly in this soil, which was rich in humus, than in most field soils, and with the large application, actually more available phosphorus was supplied for the use of the organisms.

It is interesting to consider this effect of rock phosphate on sulfofication from the practical standpoint. If raw rock phosphate will stimulate sulfate production to such a large extent as these results indicate, it may be that the material would be quite as valuable as a phosphorus *and sulfur* fertilizer, such as acid phosphate, at least on soils not extremely low in sulfur. In other words, if rock phosphate will stimulate sulfate production from soils sufficiently to supply the needs of crops, it may be unnecessary to use a special sulfur fertilizer except in extreme cases and the phosphorus fertilizer may be depended on for a dual purpose. Of course, this is assuming that the rock phosphate gives as good effects from the phosphorus standpoint as the acid phosphate, a point which is far from settled.

When gypsum was applied with the rock phosphate, increases in sulfofication were noted, but these gains were smaller than those secured with the rock phosphate alone and smaller also than those given by the gypsum alone. The increases were about the same as those given by the acid phosphate. It is apparent again, therefore, that the single constituents gave more effect than the two together. In this case also, just as with the acid phosphate, the cause for this smaller increase with the combined materials is not apparent and may be due to complicated bacterial changes where the two substances were combined. It is evident that on soils not very deficient in total sulfur, rock phosphate alone may prove just as beneficial as when applied with gypsum because of a greater production of available sulfates.

It must be emphasized again that these results apply to this particular soil only and not to soils in general. The soil used in this work was unusually high in sulfur and hence the effects of sulfur fertilizers would be less pronounced than on soils poorer in sulfur. If there are such pronounced effects on the sulfofying power of this particular soil by small applications of the various fertilizing materials, a much greater effect might be expected from the same substances on a soil poorer in sulfur, or a more normal soil.

The following conclusions, therefore, from this work seem entirely justified and while they apply specifically to this particular soil, they may be found to be of much more general application:

Applications of acid phosphate, rock phosphate, gypsum, rock phosphate and gypsum and of mono-calcium phosphate increased the sulfofying power of the soil to a considerable extent.

The rock phosphate, mono-calcium phosphate and gypsum gave the largest increases, larger than those given by the mixtures or by the acid phosphate.

Any of these materials, therefore, when applied to the soil in normal field amounts may be expected to increase sulfate production and their effects on crops grown, if any, may be due partly at least to this influence on sulfur transformation. It is particularly interesting to note the greater effect of the rock phosphate on sulfofication than of the acid phosphate. On soils not strongly depleted in sulfur, therefore, but deficient in sulfofication, and also in need of phosphorus, it seems possible that the rock phosphate would prove as satisfactory as the more soluble acid phosphate. Crop yields must, of course, prove this point before it can be accepted definitely.

No reason can be assigned for the greater effects on sulfofication of the single constituents over the combinations. They were probably due to complicated bacterial processes which the latter engendered, and about which nothing is known as yet.

THE AMMONIFICATION EXPERIMENTS

The samples drawn on the dates already mentioned were tested for their ammonifying power by the casein-fresh-soil method and the dried-blood-soil-method. The former method was employed at the first and second samplings, the incubation period being three and five days respectively, but the results were not satisfactory, and the remaining tests were made by the dried-blood method. All of the results are given in table VIII.

The duplicates were much more satisfactory where the casein was used, but the effects of the treatments were not clearly pronounced; the differences in ammonifying power of the soil were too small in many cases to be conclusive. The dried blood results were more definite, but the same difficulty which is usually met with was encountered with them, that is, the impossibility of securing entirely satisfactory duplicate determinations. However, the results given in the table show certain tendencies among the soils, and it will be worth while to call attention briefly to some points which appear more or less definitely.

The calcium sulfate had the greatest effect of any of the materials on ammonification, the mono-calcium phosphate and acid phosphate were about equal in their effect, but lower than that of the calcium sulfate and the raw rock phosphate and rock phosphate with calcium sulfate had small influences.

The stimulative action of all these materials on ammonification is very clearly shown by the results secured, and some relations are evident between the ammonification results and the sulfofication tests. Thus in both cases the calcium sulfate exerted the greatest stimulative action of any of the materials used. In the sulfofication results, however, the rock phosphate alone gave practically as large an effect as the gypsum, while in ammonification it had very much less influence.

TABLE VIII. AMMONIFICATION TESTS

Pot No.	Lab. No.	Mgs. N. as NH_3	Av. for treatment	Mgs. N. as NH_3	Av. for treatment	Mgs. N. as NH_3	Av. for treatment	Mgs. N. as NH_3	Av. for treatment	Mgs. N. as NH_3	Av. for treatment
1	1	86.08		82.49		247.2		268.2		212.4	
	2			83.45		235.4		247.5		229.9	
2	3	86.46		81.53		277.2		288.7		212.1	
	4		86.27	82.49	82.49	282.6	261.1	280.7	271.3	210.9	216.3
3	5	84.53		80.57		292.9		309.7		246.5	
	6			82.49		283.2		291.9		236.6	
4	7	83.76		83.45		292.3		309.7		211.2	
	8		84.14	83.93	82.64	281.4	287.5	294.6	301.5	238.3	233.1
5	9	88.01		83.05		292.3		300.1		213.0	
	10			83.45		284.1		295.9		237.9	
6	11	89.17		83.93		378.9		276.9		234.6	
	12		88.59	83.45	83.47	271.1	281.7	280.2	288.3	251.5	234.2
7	13	91.87		85.84		306.5		301.2		249.3	
	14			83.45		284.4		289.6		210.3	
8	15	93.03		80.09		282.9		279.7		222.9	
	16		92.45	80.09	82.36	277.2	287.8	282.8	288.3	234.3	228.9
9	17	87.62		83.05		285.1		291.9		212.4	
	18			85.84		305.6		275.6		203.7	
10	19	86.85		82.01		269.0		280.7		211.4	
	20		87.38		83.22	283.4	285.8	276.9	281.3	214.3	210.4
11	21	87.23		83.93		274.5		300.2		225.6	
	22			85.84		272.6		283.5		220.5	
12	23	87.23		85.36		235.9		293.8		217.9	
	24		87.23	87.28	85.60	230.6	257.9	253.4	282.7	210.9	218.9

Again in the sulfofication tests the acid phosphate had less effect than either the calcium sulfate or mono-calcium phosphate, while in ammonification it showed less influence than the calcium sulfate but practically the same as the mono-calcium phosphate. In both cases the mixture of rock phosphate and calcium sulfate gave small influence.

It is impossible to explain these divergencies, some of which, owing to the difficulties encountered in the methods, are not as pronounced as they should be. Indeed, it is doubtful if the present results conclusively show any definite differences in effect on ammonification among the various substances used. A large enough number of determinations was not made and the duplicate results were not in satisfactory agreement.

The stimulative action of all the substances on ammonification was clearly shown, however, just as with sulfofication, and hence there must be some relationship between the two processes. Of course, the same groups of organisms are not involved, but they may belong in the same class because of their requirements for growth, especially their food materials and the most favorable mechanical soil conditions.

The differences in the effect of phosphorus fertilizers may have been due to different effects of phosphorus as a food material on the two groups of bacteria, but these variations were not distinctive and probably the food requirements of the different groups are not very dissimilar.

THE CROP YIELDS

The oats were harvested just prior to maturity and the green and dry weights secured. The crop was analyzed for nitrogen and the removal of nitrogen from the soil in the crop was calculated. All these results are given in table IX.

All the applications of phosphorus, except the mono-calcium phosphate, increased the crop yield. The acid phosphate gave the largest increase, much larger than the raw rock. When the gypsum was applied with the rock phosphate, slightly lower yields were secured than when the rock phosphate alone was used. The difference, however, was slight and should not be considered as indicating any depression from the use of the gypsum.

The gypsum alone and the mono-calcium phosphate gave no effects. The actual average yields were slightly less than those of the check soils, but the differences in the duplicate pots were as great as those between the checks and the treated soils, and hence the results should merely indicate an absence of effect for the treatments.

It will be recalled that the soil used in this work was very low in phosphorus and hence a beneficial effect of the phosphorus

TABLE IX. THE CROP YIELDS

Pot No.	Treatment	Green weight grams	Average	Dry weight grams	Average	Per cent N. in crop	Grams N. in crop	Average
1	Check	266.0		52.9		2.572	1.3605	
2	Check	262.4	264.2	49.7	51.3	2.423	1.1942	1.2773
3	$CaSO_4$	263.55		50.45		2.310	1.1654	
4	$CaSO_4$	243.9	253.7	49.0	49.72	2.346	1.1495	1.1574
5	$CaH_4(PO_4)_2$	239.6		48.0		2.677	1.2849	
6	$CaH_4(PO_4)_2$	271.35	255.4	50.45	49.22	2.201	1.1104	1.1976
7	Acid Phosphate	327.8		62.7		2.699	1.6923	
8	Acid Phosphate	319.9	323.9	63.0	62.85	2.561	1.6134	1.6528
9	Raw Rock Phos.....	300.0		55.0		2.751	1.5121	
10	Raw Rock Phos.....	323.7	311.9	56.5	55.75	2.652	1.4984	1.5052
11	Raw Rock Phos. plus $CaSO_4$	305.3		54.9		2.959	1.6244	
12	Raw Rock Phos. plus $CaSO_4$	277.4	291.3	51.7	53.3	2.553	1.3200	1.4722

fertilizers on crop yields might have been expected. It is evident that when a soil is as low as this one was, applications of phosphorus fertilizers would prove of value. These results also indicate a superior value for the acid phosphate over the rock phosphate. No conclusions applicable to field conditions should be drawn from this single experiment, especially as it was conducted under greenhouse conditions. The results may merely serve to indicate what *may* occur under field conditions *on this particular soil type.* No attempt has been made, therefore, to calculate the relative cost of applications and the value of increases which would be necessary in field tests in order to arrive at some conclusions regarding the relative values of the applications.

Why the mono-calcium phosphate should not have brought about any increase in yield is not apparent from the results. A slight depression in the crop yield was actually observed, but it was not large enough to be distinctive as the differences in the duplicate pots were wider than the differences between the check and treated pots and it appears merely, therefore, that the plants were unable to utilize the phosphorus from this compound as well as in acid phosphate. The sulfate present in the acid phosphate could not account for the greater effect of the latter material as the sulfate alone gave no effect on the crop. Possibly the acidity of the mono-calcium phosphate may explain the results especially as this would have more effect in the absence of the calcium sulfate than where the two occur together in the acid phosphate.

The use of calcium sulfate on this soil was definitely shown to be of no value. This is as might be expected from the fact that the soil was so abnormally high in sulfur. There was evidently an abundance of sulfur present and in the presence of sufficient organic matter and lime, the process of sulfofication proceeded rapidly enough to keep the oats supplied with sulfates. Even in the presence of phosphorus, where a larger growth was secured, the sulfate had no additional effect showing the absence of any need for sulfates on this soil.

Comparing the results of the sulfofication and ammonification tests with the crop yields, it is found that there were some agreements and some discrepancies. The gypsum exerted the greatest effect on sulfofication and likewise on ammonification, but had no influence on the crop grown. Mono-calcium phosphate likewise gave a considerable increase in sulfofying power and in ammonifying power, but had no effect on the yield of oats. Acid phosphate increased sulfofication, ammonification and crop yield, the latter to the greatest extent of any material used and the two former processes to as great an extent as the other substances applied. Rock phosphate increased the crop yield and the sulfofying power of the soil, but had no pronounced effect on ammonification. It is apparent, therefore, that the sulfofying power of a soil may be increased without a corresponding increase in crop yield occurring. As has been mentioned, conclusions should hardly be drawn from the ammonification results, but it would seem that other factors might be of greater importance from the crop standpoint than the transformation of soil nitrogen, at least in greenhouse soils.

In general, these crop yields show that on this soil, rich in sulfur but poor in phosphorus, phosphate fertilizers gave a pronounced effect while sulfates had no influence. The supply of sulfur and of nitrogen available for plant growth was evidently sufficient and phosphorus was the limiting factor of growth. Hence the influence of applications of materials merely increasing the supply of nitrates and sulfates was not apparent above the effect of the use of phosphorus.

CONCLUSIONS

This experiment leads to the following conclusions:

1. The sulfate content of the soil varied only slightly from one sampling to the next. There were no sudden or striking changes in the amount of sulfates present in soil kept fallow in the greenhouse.

2. The sulfate content of soils in the field is subject to the same influences as the nitrate content, but the effects are probably much less pronounced.

3. Calcium sulfate, mono-calcium phosphate, acid phosphate, rock phosphate and rock phosphate plus gypsum increased the sulfofying power of the soil. The sulfate alone and phosphates alone had greater effects than combinations of the two materials as in acid phosphate.

4. All the materials used increased the ammonifying power of the soil, but the differences between the effects of the various substances were not

pronounced. The rock phosphate had less effect, however, than the other materials.

5. The sulfofication tests and ammonification tests did not always run parallel, although very similar effects of the materials used, on the two processes, were noted.

6. The phosphorus fertilizers except mono-calcium phosphate increased the yield of oats on the soil, the acid phosphate to a greater extent than the rock phosphate. The sulfate had no effect on the crop yield. Such results were expected on this soil rich in sulfur but deficient in phosphorus. The lack of effect from the mono-calcium phosphate was probably due to the acidity which was of more effect in the absence of the sulfate than when the two were together as in the acid phosphate.

7. The crop yields, sulfofication and ammonification results were not always parallel. In general it appeared that on this soil increases in sulfofication were not necessarily parallel with increases in yields. The ammonification results were not conclusive but indicate that materials supplying plant food constituents which are lacking in the soil may be of double value because of increases in the production of other plant food constituents in an available form.

STUDIES IN SULFOFICATION II

Series I. The Proper Incubation Period for Tests of Sulfofication

Previous tests of soils for their sulfofying power by the use of free sulfur, which was found to be the best material to use, were incubated for 10 days. It seemed desirable to ascertain whether this period allowed of the greatest differentiation between soils from different sources and under varying treatments. Shorter periods of incubation were eliminated, as less satisfactory in earlier experiments, and hence the tests here were carried out at 7, 10, 12, and 14 day periods.

Five soils, very different as to texture and composition, and thus presumably varying widely in sulfofying power, were selected. Fresh soil was used, being weighed out in 100-gram quantities in tumblers, 100 mgs. of sulfur added to each and the moisture content of each of the soils adjusted to the optimum for that particular soil. The sulfates produced at the end of the various incubation periods were determined as usual.

Examining the results given in table X, it is apparent that considerably larger amounts of sulfates were produced from the sulfur added with the longer incubation periods. None of the soils showed more than a trace of sulfates at the beginning of the experiment so the entire amount found at the end of the incubation may be considered as produced from the sulfur added.

At the end of the 7-day period, the differences between the various soils were much too small in several cases to be conclusive. After 10 days' incubation, the amounts of sulfates produced were somewhat larger and the ranking of the soils in sulfofying power had changed materially. The duplicate determinations also agreed much better. In 12 days, the differences had become still more pronounced, but the ranking of the soils was the same. After 14 days' incubation, the variations were

TABLE X. SULFATES PRESENT AFTER DIFFERENT PERIODS OF INCUBATION

No.	Soils	S. as SO_4 after 7 da.	Average	S. as SO_4 after 10 da.	Average	S. as SO_4 after 12 da.	Average	S. as SO_4 after 14 da.	Average
1	Heavy black	5.72		11.76		7.92		21.47	
	Woodland soil........	5.12	5.42	6.72	9.24	11.00	9.46	20.54	21.00
2	Typical sand	8.20		9.00		9.12		14.00	
	Riverbank in sod.....	6.68	7.44	8.56	8.78	9.52	9.32	14.40	14.20
3	Humus Plot 107......	5.84		5.00		4.88		6.10	
	Check	8.40	7.12	4.00	4.50	5.16	5.02	6.23	6.16
4	Humus Plot 101......	9.88		10.80		18.33		30.80	
	Continuous timothy..	10.28	10.08	11.44	11.12	18.67	18.50	30.60	30.70
5	Cornfield soil	9.40		6.04		7.20		10.00	
	River terrace	8.08	8.74	6.55	6.30	8.56	7.88	10.20	10.10

larger but the ranking of the soils was the same as after the 10 and 12 day periods.

These results indicate that when soils are tested for their sulfofying power by the free-sulfur-fresh-soil method the tests should be incubated for at least 10 days to secure the proper ranking of the soils. Much better results may be secured by incubating the samples for 12 or even 14 days.

Series II. The Effect of Gypsum on Sulfofication

In the earlier experiments gypsum was found to exert a stimulative effect on sulfofication, but the amounts used were rather small and further tests seemed desirable to ascertain whether large applications would show a greater effect or whether they would depress the activities of the sulfofying bacteria. This series was planned to test this point. The soil used was a Marshall silt loam from Lee county, Iowa. It was air-dried, sieved through a 20-mesh sieve and weighed out as usual. Sulfur in the usual amount and the special quantities of calcium sulfate were then added and thoroly stirred in. Ten c.c. of a soil infusion, made by shaking 100 gms. of fresh soil in 200 c.c. of water for five minutes, were added and sufficient sterile water supplied to bring the moisture content up to the optimum. The tests were then incubated for 10 days, after which the sulfates were estimated as usual.

Table XI gives the arrangement of the experiment, together with the results secured. It appears that the smallest amount of gypsum had practically no effect on the sulfofying power of the soil while the larger amounts depressed the production of sulfates. The greatest depression occurred with the use of 0.30 gm. of the sulfate and when 0.50 gm. was added, the depression was less but still greater than with the 0.10 gm. of the sulfate.

The previous experiments, which showed the stimulating effect of gypsum, were carried out in greenhouse soils and much

TABLE XI. THE EFFECT OF $CaSO_4$ ON SULFOFICATION

No.	Treatment	Mgs. S. as SO_4 after Incubation	Mgs. S. as SO_4 added in $CaSO_4$	Mgs. S. as SO_4 from free S.	Av. Percent S. Sulfofied
1	Nothing	21.0	0	21.0	
2	Nothing	21.6	0	21.6	21.3
3	0.05 gms. $CaSO_4$.....	28.4	9.35	19.05	
4	0.05 gms. $CaSO_4$.....	30.6	9.35	21.25	20.15
5	0.075 gms. $CaSO_4$.....	33.0	14.02	18.98	
6	0.075 gms. $CaSO_4$.....	32.0	14.02	17.98	18.48
7	0.10 gms. $CaSO_4$.....	37.6	18.69	18.91	
8	0.10 gms. $CaSO_4$.....	36.4	18.69	17.71	18.31
9	0.30 gms. $CaSO_4$.....	62.8	56.07	6.73	
10	0.30 gms. $CaSO_4$.....	60.0	56.07	3.93	5.33
11	0.50 gms. $CaSO_4$.....	106.0	93.45	12.55	
12	0.50 gms. $CaSO_4$.....	110.0	93.45	16.55	14.55

smaller amounts were used than was the case here, so that these later results are not in any way opposed to the earlier. It was apparent in those results that applications of gypsum at the rate usually employed in field soils stimulated sulfofication and hence it is evident that the application of gypsum cannot be increased to any appreciable extent without bringing about a depression in sulfofying power.

There could be no practical value, therefore, in making heavy applications of gypsum to increase sulfofication. Of course, these results should not be accepted as conclusive for field practice because of the fact brought out in earlier work that gypsum is rather readily assimilated in the soil. There was probably some assimilation in these experiments and the results for the treated soils may have been too small. It was impossible to ascertain the extent of the assimilation and in making the calculations the total amount of sulfate added was subtracted from the final figure.

It is safe to conclude, however, that the most effective applications of gypsum, economically, are those commonly employed in field practice.

Series III. The Effect of Calcium Carbonate on Sulfofication

If sulfofication is an important process in field soils as it seems, the effect on it of applications of calcium carbonate must be considered. Is it increased, as are ammonification and nitrification, or is it decreased when the acidity of the soil is remedied by the use of limestone? This test was planned to throw some light on this point.

The same soil as used in the preceding test was employed here. The soil was weighed out, the calcium carbonate in special amounts, and the sulfur added and stirred in, 10 c.c. of a fresh soil infusion applied, the moisture content adjusted to the optimum and the tests incubated for 10 days. The results of the sulfate determinations appear in table XII. It is clear that the use of calcium carbonate on an acid soil increased sulfofication.

TABLE XII. THE EFFECT OF $CaCO_3$ ON SULFOFICATION

No.	Treatment	Mgs. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	21.0	
2	Nothing	21.9	21.4
3	Neutralized	27.2	
4	Neutralized	25.6	26.4
5	Neutralized plus 0.1 gm. $CaCO_3$	32.2	
6	Neutralized plus 0.1 gm. $CaCO_3$	32.8	32.5
7	Neutralized plus 0.3 gm. $CaCO_3$	40.6	
8	Neutralized plus 0.3 gm. $CaCO_3$	36.4	38.5
9	Neutralized plus 0.5 gm. $CaCO_3$	32.6	
10	Neutralized plus 0.5 gm. $CaCO_3$	33.4	33.0
11	Neutralized plus 1.0 gm. $CaCO_3$	35.0	
12	Neutralized plus 1.0 gm. $CaCO_3$	33.4	34.2
13	Neutralized plus 5.0 gm. $CaCO_3$	32.6	
14	Neutralized plus 5.0 gm. $CaCO_3$	30.4	31.5

There was a considerable increase when the acidity of the soil was neutralized and with further additions of calcium carbonate still greater gains in sulfofication were found. The greatest gain, however, occurred with the use of 0.3 gm. per 100 gms. of soil, corresponding to 6,000 lbs. per acre and beyond that point the increases were somewhat less.

If the applications of the carbonate had been increased still further, it is possible that the sulfofication would have decreased below that in the soil with its acidity just neutralized or even below the acid soil. But the amounts used here were not sufficiently large to bring about such a decrease and, inasmuch as the applications made in the field never exceed the amounts used here, there need be no apprehension of decreasing sulfofication by the use of ordinary amounts of calcium carbonate to remedy acid conditions in the soil.

It is also evident from these results that calcium carbonate up to 6,000 lbs. per acre increased the sulfofying power of this soil. Larger amounts, such as are rarely used in practice, gave considerable increase in sulfofication, but these were somewhat less than those secured with the three ton amount.

Series IV. The Effect of Magnesium Carbonate on Sulfofication

Having ascertained that calcium carbonate exerted a beneficial effect on sulfofication, it was deemed desirable to determine whether magnesium carbonate would have the same effect or not.

The soil used in this series was the same as in the previous series. The arrangement of the test was also the same, except that no acid soil was incubated and that magnesium carbonate was added in place of calcium carbonate. The check soils in this case were neutralized with calcium carbonate and all the other soils received additional amounts of magnesium carbonate.

TABLE XIII. THE EFFECT OF $MgCO_3$ ON SULFOFICATION

No.	Treatment	Mgs. S. as S. after Incubation	Average for Treatment
1	Nothing	24.4	
2	Nothing	25.7	25.0
3	0.1 gm. $MgCO_3$	28.0	
4	0.1 gm. $MgCO_3$	31.4	29.7
5	0.3 gm. $MgCO_3$	26.5	
6	0.3 gm. $MgCO_3$	21.4	23.9
7	0.5 gm. $MgCO_3$	19.6	
8	0.5 gm. $MgCO_3$	17.1	18.3
9	1.0 gm. $MgCO_3$	17.0	
10	1.0 gm. $MgCO_3$	16.5	16.7
11	5.0 gm. $MgCO_3$	15.8	
12	5.0 gm. $MgCO_3$	15.1	15.4

Soil neutralized with $CaCO_3$.

Table XIII, which gives the results, shows that the smallest amount of magnesium carbonate increased slightly the sulfofying power of the soil, but the larger amounts gave gradually increasing depressions up to the largest amount employed here. It is evident that applications of magnesium carbonate in amounts greater than 2,000 lbs. per acre depressed the sulfofying power of this soil below that shown by the sample receiving no magnesium carbonate at all.

Comparing these results with those secured with the calcium carbonate in the previous test, it is found that the use of magnesium carbonate at the rate of 2,000 lbs. per acre gave less effect on sulfofication than the use of the same amount of calcium carbonate, both additions being made to a neutralized soil.

While, however, the use of 3 tons of calcium carbonate per acre above that necessary to neutralize the acidity of the soil, increased the sulfofying power of the soil, the application of that much magnesium carbonate depressed sulfofication considerably.

It is apparent, therefore, that the application of magnesium carbonate to neutral soils should be made with care, and amounts greater than two tons per acre might depress the sulfofying power of the soil.

Evidently the sulfofying bacteria are much less sensitive to the presence of an abundance of calcium carbonate than to the presence of much magnesium carbonate. This is in accord with other bacteriological results dealing with the transformation of soil nitrogen, and it also is in accord with many crop results.

Series V. The Effect of Calcium and Magnesium Carbonates on Sulfofication

This test was planned to determine the effect of calcium and magnesium carbonates combined on sulfofication. The same soil and the same arrangement of the experiment was used here as in the two previous tests, except that both calcium and magnesium carbonates were applied. The amounts of these materials combined were the same as the amounts of the single substances used in the earlier series.

TABLE XIV. THE EFFECT OF $CaCO_3$ AND $MgCO_3$ ON SULFOFICATION

No.	Treatment	Mgs. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	27.0	
2	Nothing	24.8	25.9
3	0.05 gms. $CaCO_3$ plus 0.05 gms. $MgCO_3$	28.0	
4	0.05 gms. $CaCO_3$ plus 0.05 gms. $MgCO_3$	20.0	29.0
5	0.15 gms. $CaCO_3$ plus 0.15 gms. $MgCO_3$	25.7	
6	0.15 gms. $CaCO_3$ plus 0.15 gms. $MgCO_3$	24.1	24.9
7	0.25 gms. $CaCO_3$ plus 0.25 gms. $MgCO_3$	18.2	
8	0.25 gms. $CaCO_3$ plus 0.25 gms. $MgCO_3$	21.4	19.8
9	0.50 gms. $CaCO_3$ plus 0.50 gms. $MgCO_3$	16.1	
10	0.50 gms. $CaCO_3$ plus 0.50 gms. $MgCO_3$	16.4	16.2
11	2.50 gms. $CaCO_3$ plus 2.50 gms. $MgCO_3$	19.8	
12	2.50 gms. $CaCO_3$ plus 2.50 gms. $MgCO_3$	22.3	21.0

The results of the tests appear in table XIV. The use of calcium and magnesium carbonates in amounts larger than 2,000 lbs. per acre of both together depressed the sulfofying power of this soil below that of the neutral soil. The check soils here represented the soil with its entire acidity neutralized with calcium carbonate. Increasing the application of calcium and magnesium carbonates together beyond 6,000 lbs. per acre decreased the sulfofying power of this soil, the depression increasing with increasing amounts applied.

It is apparent, therefore, that on this soil applications of calcium carbonate gave greater effects on sulfofication than the use of magnesium carbonate or combinations of the two carbonates and that the use of magnesium or dolomitic limestones on this soil, after its acidity has been neutralized with calcium carbonate, may lead to a depression in sulfofying power if the amounts used exceed 2,000 lbs. per acre. Non-magnesian limestones, on the other hand, up to 6,000 lbs. per acre increased the sulfofying power of the soil, and in larger applications, gave smaller effects on sulfofication, but no actual depressions.

CONCLUSIONS

These tests on this soil lead to the following conclusions:

1. In the use of the free-sulfur-fresh-soil method for testing the sulfofying power of soils, the incubation period should be fourteen days at room temperature to give the most conclusive results. Ten days' incubation gave the relative sulfofying powers of soils quite accurately, but the differences were much more distinctive for the longer period.

2. Calcium sulfate in ordinary applications had no detrimental effect on sulfofication, but very large applications might decrease the rate of oxidation of sulfur.

3. Calcium carbonate in ordinary applications on acid soils increased sulfofication considerably and even in excessive amounts affected sulfur oxidation favorably.

4. Magnesium carbonate in small amounts increased sulfofication, but in large amounts depressed it even below that in the same soil with its acidity unneutralized.

5. Magnesium carbonate and calcium carbonate in combination exerted a beneficial influence on sulfofication when used in small amounts. Larger applications, however, depressed the oxidation of sulfur. The effects of the combined materials were less than that of the calcium carbonate alone.